Oxidation Of Di-Peptides With Mn(III): Synthesis, Characterization And Mechanistic Study

Article *in* Reaction Kinetics and Catalysis Letters · January 2001

DOI: 10.1023/A:1010507301254

4 authors, including:

M.N. Kumara
chemistry Department
54 PUBLICATIONS **160** CITATIONS
SEE PROFILE

Channe D Gowda
University of Mysore
353 PUBLICATIONS **7,089** CITATIONS
SEE PROFILE

Jointly published by
Kluwer Academic Publishers, Dordrecht
and Akadémiai Kiadó, Budapest

React.Kinet.Catal.Lett.
Vol. 72, No. 2, 331-342
(2001)

RKCL3724

OXIDATION OF DI-PEPTIDES WITH Mn(III): SYNTHESIS, CHARACTERIZATION AND MECHANISTIC STUDY

Manikyanahally N. Kumara, Dase Gowda Channe Gowda and Kanchugarakoppal S. Rangappa*
Department of Studies in Chemistry, University of Mysore, Mysore-570006, India

Received May 2, 2000
In revised form February 22, 2001
Accepted March 6, 2001

Abstract

Synthesis and characterization of the dipeptides(DP) namely Glycyl-Proline (Gly-Pro), Alanyl-Proline (Ala-Pro) and Valyl–Proline (Val-Pro), were made. Kinetics of oxidation of these DP by Mn(III) have been studied in the presence of sulfate ions in acidic medium at 26°C. The reaction was followed spectrophotometrically at λ_{max} = 500 nm. A first order dependence of rate on both Mn(III) and DP was observed. The rate is independent of the concentration of reduction product, Mn(II) and hydrogen ions. Effects of varying dielectric constant of the medium and addition of anions such as sulfate, chloride and perchlorate were studied. Activation parameters have been evaluated using Arrhenius and Eyring plots. The oxidation products were isolated and characterized. A mechanism involving the reaction of DP with Mn(III) in the rate limiting step is suggested.

Keywords: Oxidation, dipeptide, Mn(III)

INTRODUCTION

There has been a great deal of attention focussed on the oxidation of organic substrates by high-valent metal ions. Of these, manganese(III) oxidation is of special importance due to their biological relevance [1]. Manganese(III) porphyrins have been studied as possible models for closely related biologically significant systems [2]. Some studies have been reported on the kinetics of

* Present address**:** Prof. K.S. Rangappa, DOS in Chemistry, University of Myrore, Manasagangotri, Mysore-570006, India

0133-1736/2001/US$ 12.00.

manganese(III) oxidation of various substrates in perchlorate, sulfate, acetate and pyrophosphate media [3-5]. Peptides and proteins are the most characteristic chemical compounds found in living cells. They are composed of amino-acids. The peptides like enkephalins, oxytocin, vasopressin, LHRH, opioid peptides play a very important role in biology. These peptides are susceptible to enzymes. Extensive work has been reported on the enzymatic degradation of these peptides. Although the kinetics of oxidation of amino acids with various metal ions and by halogens [6-10] in acid and alkaline media have been studied, oxidation of these biologically active peptides have not been reported in literature.

We have synthesised there DP *viz*. Glycyl-Proline (Gly-Pro), Alanyl-Proline (Ala-Pro) and Valyl-Proline (Val-Pro), to study the kinetics of oxidation with Mn(III) in acid medium to elucidate the mechanism of these redox reactions.

EXPERIMENTAL

A 0.05 mol dm^{-3} solution of manganese(III) sulfate was prepared by the electrolytic oxidation of Mn(II) sulfate in aqueous sulfuric acid by the procedure reported [11] previously. Even though the solution appeared to be stable for more than one month at $[H^+] > 5.0$ mol dm^{-3}, it was prepared afresh daily. All other reagents were prepared from AR grade chemicals.

Preliminary studies

The maximum absorption, λ_{max} of manganese(III) sulfate solution occurs at 500 nm. The standard redox potential E_0^1 of Mn(III)/Mn(II), the oxidizing power of the oxidant, generally decreases on complexation. The standard redox potential was measured at the specified experimental conditions. These details are reported previously [11], the formal redox potential E_0^1 obtained at different $[H_2SO_4]$ and in presence of the complexing agents HSO_4^-, $P_2O_7^{4-}$ and F^- are 1.51, 1.48 and 1.42 V, respectively. Triply distilled water was used for preparing aqueous solutions.

Peptides synthesis

General

All the amino acids used except glycine are of L-configuration unless otherwise specified. All *tert*-butyloxycarbonyl (Boc) amino acids, amino acid derivatives, 1-ethyl-3(3-dimethylaminopropyl)carbodiimide (EDCI), 1-hydroxybenzotriazole (HOBt), trifluroacetic acid (TFA) and N-

methylmorpholine (NMM) were purchased from Advanced Chem. Tech. (Louisville, Kentucky, USA). All solvents and reagents were of analytical grade or were purified according to procedures recommended for peptide synthesis [12]. Thin layer chomatography (TLC) was carried out on silica gel plates obtained from Whatman Inc., with the following solvent systems: chloroform-methanol-acetic acid (95:5:3), R_f^1 ; chloroform-methanol-acetic acid (90:10:3), R_f^2; and chloroform-methanol-acetic acid (85:15:3), R_f^3.

The compounds on TLC plates were detected by UV light after spraying with ninhydrin or by chlorine/toluidine.

Boc-Gly-Pro-OBzl

Boc-Gly (3.5 g, 0.02 mol) and HOBt (3.37 g, 0.022 mol) in DMF (30 mL) was cooled to –15 °C and EDCI (4.21 g, 0.022 mol) was added. After stirring for 20 mins, a precooled solution of Pro-OBzl.HCl (4.88 g, 0.02 mol) and NMM (2.4 mL, 0.022 mol) in DMF (30 mL) was added and stirred overnight at room temperature. After evaporating DMF, the residue was taken into chloroform and extracted with 10% citric acid and 5% sodium bicarbonate solutions. The solvent was removed under reduced pressure and recrystallized from ether/petroleum to obtain 6.38 g (yield 87.5%) of Boc-Gly-Pro-OBzl. R_f^1 0.52, R_f^2 0.74 and R_f^3 0.88.

Gly-Pro

Boc-Gly-Pro-OBzl (5.4 g, 0.015 mol) was saponified in methanol (50 mL) using 1N NaOH (2.0 equiv.) for 2 h at room temperature. After evaporating the solvent under reduced pressure, the residue was taken up in water and washed with chloroform (3x25 mL). The aqueous layer was cooled and neutralized with cold 1N HCl and extracted with chloroform (40 mL). The organic phase was washed with cold 0.1N HCl, 50% saturated NaCl and dried over Na_2SO_4. The solvent was removed in vacuum and triturated with ether, filtred, washed with ether and dried to obtain 3.9 g (yield 96.1%) of Boc-Gly-Pro. R_f^2 0.42 and R_f^3 0.36.

Boc-Gly-Pro-OH (3.8 g, 0.014 mol) was deblocked with TFA (32 mL) for 40 min. The solvent was removed under reduced pressure, triturated with ether, filtred, washed with ether to obtain TFA. Gly-Pro-OH (yield 100%).

Boc-Ala-Pro-OBzl

Boc-Ala-OH (4.1 g, 0.02 mol) was coupled to Pro-OBzl.HCl (4.8 g, 0.02 mol), using EDCI with HOBt and in the presence of NMM. The reaction was

worked up the same as Boc-Gly-Pro-OBzl to obtain 6.8 g (yield 88.2%) of Boc-Ala-Pro-OBzl. R_f^1 0.89 and R_f^2 0.78.

Ala-Pro

Boc-Ala-Pro-OBzl (5.8 g, 0.015 mol) was saponified in methanol (50 mL) using 1N NaOH for 2 h at room temperature and worked up the same as Boc-Gly-Pro-OBzl to obtain 4.2 g (yield 93.7%) of Boc-Leu-Pro-OH. R_f^2 0.24 and R_f^3 0.29. This was deblocked with TFA for 40 min to obtain TFA.Ala-Pro-OH (yield 100%).

Boc-Val-Pro-OBzl

Boc-Val-OH.H_2O (4.3 g, 0.02 mol) was coupled to Pro-OBzl.HCl. (4.8 g, 0.02 mol) using EDCI with HOBt and in the presence of NMM. The reaction was worked up the same as Boc-Gly-Pro-OBzl to obtain 6.6 g (yield 81.2%) of Boc-Val-Pro-OBzl. R_f^1 0.84 and R_f^2 0.75.

Val-Pro

Boc-Val-Pro-OBzl (6.1 g, 0.015 mol) was saponified in methanol (50 mL) using 1N NaOH for 2 h at room temperature and worked up the same as Boc-Gly-Pro-OBzl to obtain 4.4 g (yield 93.5%) of Boc-Val-Pro-OH. R_f^2 0.29 and R_f^3 0.33. This was deblocked with TFA for 40 min to obtain TFA.Val-Pro-OH (yield 100%).

Kinetic measurements

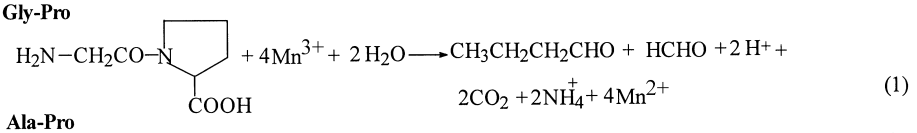
Mixtures of solution containing requisite amounts of DP, sulphuric acid (to maintain known acid concentration), manganese(II) and water (to keep the total volume constant) were taken in stopperd boiling tube. The mixture was thermally equilibrated in a water bath at 26°C. To the solution in this tube, was added an aliquot of pre-equilibrated manganese(III) sulfate stock solution to give a known overall concentration. The progress of the reaction was monitored for two half-lives by measuring the absorbance of unreacted Mn(III) at 500 nm using a spectrochem MK II. Spectrometer. The reaction mixture was quenched appropriately. Plots of log (absorbance) *vs* time were linear. The rate constants, k_{obs}, calculated from these plots were reproducible within ± 3% error.

Stoichiometry

The mixtures containing DP (0.001 mol dm^{-3}), acid (0.1 mol dm^{-3}) and excess Mn(III) (0.005 mol dm^{-3}) were kept for 24 h at 26°C. The unconsumed Mn(III) was then determined. Four mols of oxidant were sufficient to oxidise one mole of DP leading to aldehydes, carbon dioxide, ammonia, Mn(II) and hydrogen ion.

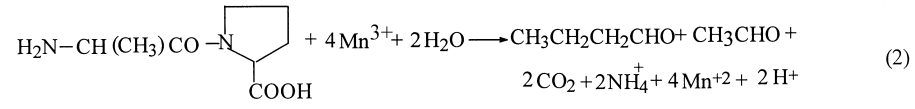
Based on the experimental results, the following stoichiometric equations are valid.

Gly-Pro



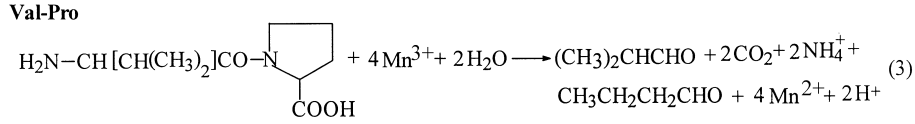
$$H_2N-CH_2CO-N(COOH) + 4Mn^{3+} + 2H_2O \longrightarrow CH_3CH_2CH_2CHO + HCHO + 2H^+ + 2CO_2 + 2NH_4^+ + 4Mn^{2+} \quad (1)$$

Ala-Pro



$$H_2N-CH(CH_3)CO-N(COOH) + 4Mn^{3+} + 2H_2O \longrightarrow CH_3CH_2CH_2CHO + CH_3CHO + 2CO_2 + 2NH_4^+ + 4Mn^{+2} + 2H^+ \quad (2)$$

Val-Pro



$$H_2N-CH[CH(CH_3)_2]CO-N(COOH) + 4Mn^{3+} + 2H_2O \longrightarrow (CH_3)_2CHCHO + 2CO_2 + 2NH_4^+ + CH_3CH_2CH_2CHO + 4Mn^{2+} + 2H^+ \quad (3)$$

Product analysis

After the reaction was completed, the reaction products were extracted with ether and subjected to column chromatography on silica gel (60-200 mesh) using gradient elution (dichloromethane to chloroform). After initial separation, the products were further purified by recrystallization. Aldehydes were quantified in the ether extract by the formation of 2,4-dinitrophenyl hydrazone derivative isolable up to 95% yield. Ammonia and CO_2 were detected by the conventional tests.

RESULTS

Dependence of rate on [manganese(III)] and [DP]

All kinetic runs were performed under pseudo-first order conditions of [DP] >> [Mn(III)]. Plots of log [Mn(III)] *vs* time were linear even beyond 75% of the reaction, showing first order dependence of the rate on [Mn(III)] (Table 1). At constant $[Mn(III)]_o$, $[Mn(II)]_o$, $[H_2SO_4]$, $[Na_2SO_4]$ and temperature, the rate

increased with increase in $[DP]_o$ (Table 1). Plots of $\log k_{obs}$ *vs* $\log [DP]_o$ (Fig. 1) were linear with slopes of 1.00, 0.99 and 0.97 for Gly-Pro, Ala-Pro and Val-Pro respectively.

Table 1

Effect of reactant concentration on the rate[a]

Mn(III)x10^3 (mol dm^{-3})	[DP]x10^2 (mol dm^{-3})	Gly-Pro k_{obs} x10^4(s^{-1})	Ala-Pro k_{obs} x10^4(s^{-1})	Val-Pro k_{obs} x10^4(s^{-1})
0.6	1.0	1.86	1.12	0.88
0.8	1.0	1.86	1.12	0.88
1.0	1.0	1.86	1.12	0.88
1.2	1.0	1.86	1.12	0.88
1.4	1.0	1.86	1.12	0.88
1.6	1.0	1.86	1.12	0.88
1.8	1.0	1.86	1.12	0.88
1.0	0.6	1.12	0.67	0.53
1.0	0.8	1.48	0.89	0.70
1.0	1.0	1.86	1.12	0.88
1.0	1.2	2.24	1.35	1.04
1.0	1.4	2.60	1.56	1.20
1.0	1.6	2.04	1.77	1.38
1.0	1.8	3.36	1.99	1.54

[a]$[H_2SO_4]$ = 0.1 mol dm^{-3}, $[Mn(II)]_o$ = 0.01 mol dm^{-3}, at 26°C

Dependence of rate on [acid]

Kinetic measurements were performed in H_2SO_4 - $NaHSO_4$ solution of different [H^+]. The effective [H^+] used was evaluated with the acid of standard curve [13] of $[H_2SO_4]$ *vs* [H^+]. Increase in [H^+] (0.06 to 1.8 mol dm^{-3}), had no effect on the rate.

Dependence of rate on Mn(II) and added salts

The effect on the rate of varying concentration of Mn(II) (which is the reduction product of the oxidant) was investigated. Increase in [Mn(II)] (0.006 to 0.016 mol dm^{-3}) had no effect on the rate. Similarly the effect of anions Cl^- (0.001 to 0.1 mol dm^{-3}), SO_4^{2-} (0.001 to 0.1 mol dm^{-3}) and ClO_4^- (0.001 to 0.1 mol dm^{-3}) on the rate were insignificant. The reaction product Mn(II) had no effect on the reaction, indicating that the product is not involved in a pre-equilibrium with the oxidant.

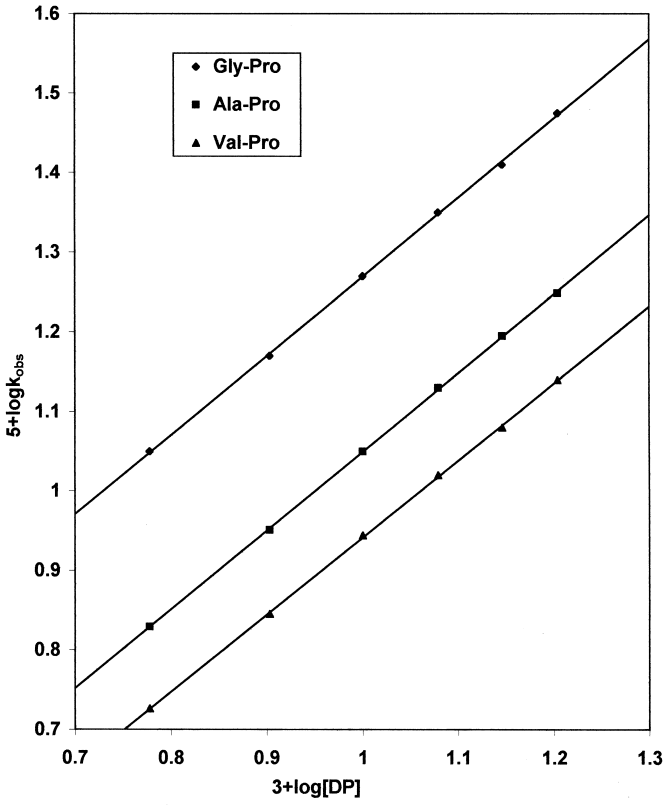


Fig. 1. Effect of [DP] on the rate at 26°C

Effect of solvent composition

The solvent composition of the medium was varied by adding methanol (0.0 to 40%) to the reaction mixture. The rate increase with increase in methanol content. The plots of log k_{obs} *vs* 1/D (D=dielectric constant of the medium) were linear with positive slopes. However, methanol oxidation did not take place under the present conditions.

Activation parameters

To determine the activation parameters, the reactions were carried out at different temperatures (21-41°C). The Arrhenius plots of log k_{obs} *vs* 1/T (Fig. 2), were found to be linear. The activation energies (Ea) were calculated from the

slope of the plots. From this value, the activation parameters $\Delta H^{\#}$, $\Delta S^{\#}$, $\Delta G^{\#}$ and the frequency factor (logA) (Table 2), were evaluated.

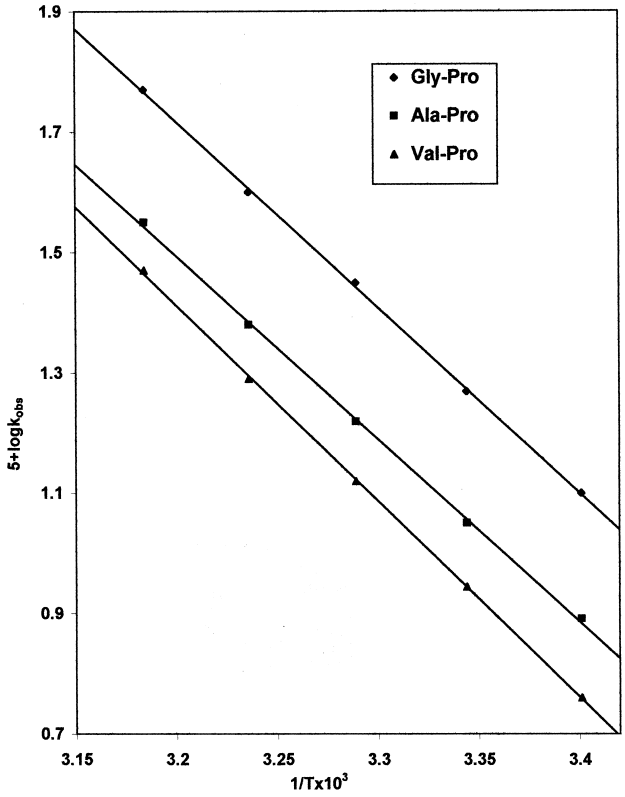


Fig. 2. Effect of temperature

Test for free radicals

Addition of aqueous solution of acrylamide to the reaction mixture did not cause polymerization suggesting the absence of free-radical involvement during the oxidation.

DISCUSSION

Data published by Diebler and Sutin [14] and Packler and Chawl [15] and Wells [16], have shown that in the presence of F^- ions, aqueous solutions of Mn(III) consist of hexaaquamanganese(III), $Mn(III)_{(aq)}$, $hydroxopenta_{(aq)}$,

$MnOH^{2+}_{(aq)}$ and $MnF^{2+}_{(aq)}$. On the same line it can be assumed with justification that Mn(III) species present in sulfuric acid medium are $Mn(III)_{(aq)}$, $MnOH^{2+}_{(aq)}$ and $MnSO_4^+$. Therefore it was shown [17] that manganese(III) sulfate in aqueous sulfuric acid contains $Mn^{3+}_{(aq)}$ and $Mn(OH)^{2+}_{(aq)}$ as reactive species.

Table 2

Activation parameters

	k_{obs} x $10^4(s^{-1})$						
T(K)	Gly-Pro	Ala-Pro	Val-Pro		Gly-Pro	Ala-Pro	Val-Pro
294	1.26	0.77	0.57	Ea (kJ mol^{-1})	59.99	58.01	63.14
299	1.86	1.12	0.88	$\Delta H^{\#}$ (kJ mol^{-1})	56.77	55.48	60.61
304	2.82	1.67	1.32	$\Delta S^{\#}$ ($JK^{-1}mol^{-1}$)	-126.47	-135.00	-120.22
309	3.98	2.40	1.95	$\Delta G^{\#}$ (kJ mol^{-1})	95.22	96.52	97.18
314	5.90	3.55	2.95	LogA	6.6	6.1	6.9

[a]$[Mn(III)]_o$ =0.001 mol dm^{-3}, $[DP]_o$ = 0.01 mol dm^{-3}, $[H_2SO_4]$ = 0.1 mol dm^{-3}

.

$$Mn^{3+}_{(aq)} + H_2O \;\Delta\; Mn(OH)^{2+}_{(aq)} + H^+ \qquad (4)$$

The hydrolysis constant calculated was k_h = 0.93±0.03. The absorption spectra of both $Mn^{3+}_{(aq)}$ and $Mn(OH)^{2+}_{(aq)}$ have been reported to be similar in both the visible and UV-region. Our observation of the electronic absorption spectra is consistent with the values reported. Kinetic studies have shown that $Mn^{3+}_{(aq)}$ is more reactive.

Formation of dihydroxo species $Mn(OH)^{2+}$ produced by further hydrolysis of $MnOH^{2+}$ is another possibility [18,19].

$$MnOH^{2+}_{(aq)} + H_2O \;\forall\; Mn(OH)_2^+ + H^+ \qquad (5)$$

Fresh solutions of manganese(III) sulfate were always prepared and used immediately after cessation of the electrolysis, thereby eliminating any reaction due to $Mn(OH)_2^+$. Molar absorption co-efficient, ε are between 131-110 dm^3 mol^{-1} cm^{-1} at [H^+] =1.20-2.50 (mol dm^{-3}). The high value of ε has been attributed to the presence of hydrolyzed species $Mn(OH)^{2+}$. Since there is no hydrogen ion dependence on the rate suggest that $Mn(OH)^{2+}_{(aq)}$ or $Mn(OH)^+_2$ are not the reactive species. Absence of sulfate effect on the reaction rate indicates that $MnSO_4^+{}_{(aq)}$ is not the active species in the present conditions. Therefore $Mn^{3+}_{(aq)}$ is the reactive species as shown in the mechanism.

The following scheme 1 accounts for the observed experimental results:

$$Mn^{+3}_{(aq)} + DP \xrightarrow{k_1} X \qquad \text{(i) rds}$$

$$X \xrightarrow{k_2} \text{Products} \qquad \text{(ii) fast}$$

Scheme 1

Hence,

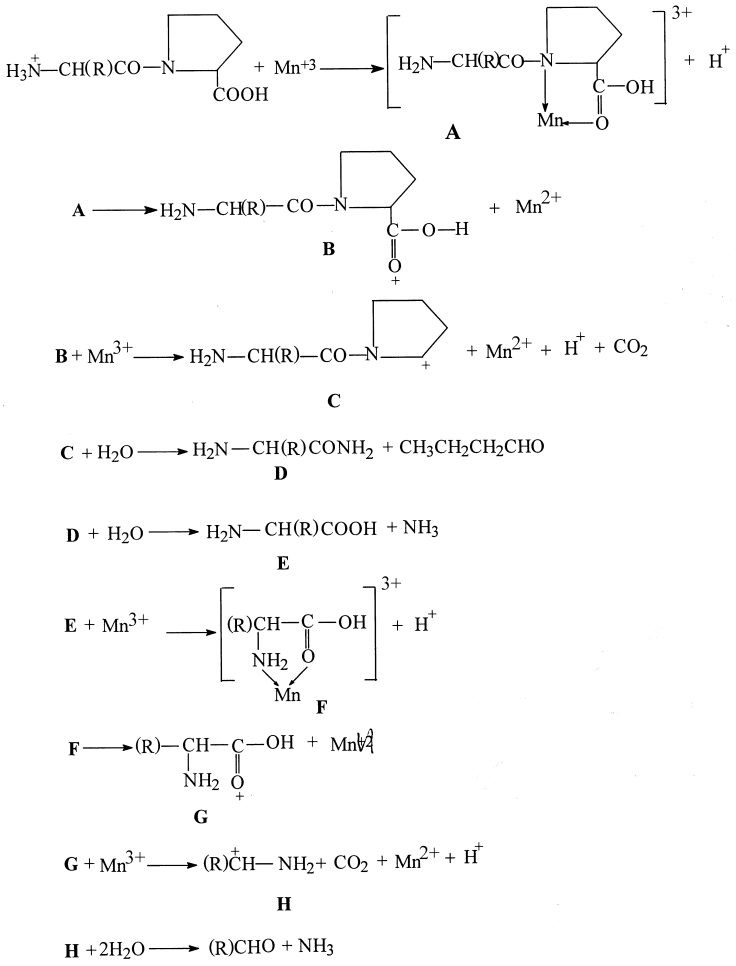
$$\text{rate} = k_1 [Mn^{3+}] [DP] \qquad (6)$$

Amis [20] has shown that a plot of $\log k_{obs}$ *vs* 1/D gives a straight line with a positive slope for positive ion-dipole interaction. The positive dielectric effect in the present investigation shows charge dispersal in the transition state, pointing towards a positive ion-dipole reaction and hence supports Scheme 2.

Spectral evidence for the formation of DP-Mn(III) complex (x)

The study of UV-visible spectra separately of pure Mn(III), DP (Gly-Pro, Ala-Pro and Val-Pro) and mixture of Mn(III) and DP showsn deviation in peak wavelength and absorbance as follows. It is noted that the values for the complex are different from each of the substrate and Mn(III) in its pure form.

Substrate	λ max (nm)	Absorbance	Complex	λ max (nm)	Absorbance
Mn(III)	500	0.97	Mn(III) + Gly-Pro	445	2.68
Gly-Pro	235	2.44	Mn(III) + Ala-Pro	433	2.65
Ala-Pro	228	2.47	Mn(III) + Val-Pro	438	2.66
Val-Pro	229	2.45			



R = H for Gly-Pro, R = CH_3 - for Ala-Pro and R = $(CH_3)_2CH$- for Val-Pro.

Scheme 2

Acknowledgement. One of the authors (K.S.R) is thankful to Department of Science and Technology (DST), New Delhi, for financial support (SP/SI/GO5/97).

REFERENCES

1. J. Boucher: *Coord. Chem. Rev.,***7**, 289 (1972).
2. M. Carlvin: *Pure Appl. Chem*., **15,** 1 (1965).
3. G. Davies: *Coord. Chem. Rev*., **4**, 199 (1969).
4. K.S. Rangappa, S. Chandraju, N.M. Made Gowda: *Synth. React. Inorg. Met–Org. Chem*., **28,** 275 (1998).
5. K.S. Rangappa, S. Chandraju, N.M. Made Gowda: *Int. J. Chem. Kinet*., **30**, 7 (1998).
6. Kanchugarakoppal S. Rangappa, Huchappa Manjunathaswamy, Manikanahally P. Raghavendra, D. Channe Gowda: *Carbohydrate Research*, **307**, 253 (1998).
7. Kanchugarakoppal S. Rangappa, Huchappa Manjunathaswamy, Manikanahally P. Raghavendra, Dandinasivara S. Mahadevappa, Dodde Gowda Channe Gowda: *J. Org. Chem.,* **63**, 531 (1998).
8. K.S.Rangappa, M.P.Raghavendra, D.S.Mahadevappa: *J. Carbohydrate Chemistry*, **16**, 351 (1997).
9. D.S. Mahadevappa, S. Ananda, N.M .Made Gowda, K.S. Rangappa: *J. Chem. Soc. Perkin Trans*., **11**, 39 (1985).
10. Kanchugarakoppal S. Rangappa, Sidde Gowda Chandraju, Dandinasivara S. Mahadevappa: *Transition Met. Chem*., **21**, 529 (1996).
11. KAMALUDDIN: *Indian J. Chem*., **19A**, 431 (1980).
12. D. Andreu, R.B. Merrifield, H. Steiner, H.G. Boman: *Proc. Natl. Acad. Sci.,***80**, 6475 (1983).
13. I.Pinto, B.S.Sherigara, H.V.K.Udupa: *Bull. Chem. Soc. Jpn*., **63**, 3625 (1982).
14. M. Diebler, Sutin: *J. Phys. Chem*., **68**, 174 (1964).
15. J.P. Pacjker, I.D. Chawla: *Inorg. Chem Soc*., **38**, 1130 (1964).
16. C.F. Wells, G.O. Davies: *J. Chem. Soc.,***(A),** 1858 (1967).
17. B.S.Sherigara, K.I. Bhat, I. Pinto, N.M. Made Gowda: *Int. J. Chem. Kinet*., **27**, 675 (1995).
18. C.F. Wells, G.O. Davies: *Nature*., **205**, 682 (1965).
19. P.A. Siskos, N.C. Peterson, R.E. Huie: *Inorg. Chem.,* **23**, 1134 (1984).
20. Amis: *J. Chem. Educ*., **30**, 351 (1953).